

THE INFLUENCE OF SUCROSE ON TISSUE CULTURES OF *ONCIDIUM GOLDIANA*, *DENDROBIUM ALICE SPALDING* AND *ARANTHERA BEATRICE NG*

by

*IRAWATI

INTRODUCTION

Tissue culture today plays an important role in the production of orchid clones throughout the world. This technique applied to orchid plants by Morel (1960) is still being perfected by other research workers. Research workers have used a wide range of media for their cultures but the perfect medium for each stage of orchid culture has still to be found.

This work is done to determine the beneficial or detrimental effects of sucrose in the culture medium.

LITERATURE REVIEW

Many orchid hybrids have been successfully propagated by the tissue culture technique and various media have been used.

In most media, sugar (sucrose) is the source of energy. However, Kunisaki et. al. (1972) found that proliferating bodies and greener cultures of *Vanda* were obtained in medium devoid of sucrose. Singh (1976) mentions that in media containing lower concentration of sucrose and naphthalene-acetic-acid, growth was better than in media which contained the higher concentrations. Teo et. al. (1973), reports that better proliferation and greener tissues of strap-leafed *Vanda* were obtained on media devoid of sucrose but which had 15% by volume of coconut water incorporated in it. Hildebrandt and Riker (1953) working with callus tissue of marigold, sunflower and tobacco, found that 2 percent of sucrose was most beneficial for the tissue. Valmayor (1974) working on orchid embryo culture used 2 percent sucrose while Gamborg (1975) suggest a concentration of 2-4 percent.

Singh (1976) working with a range of media, observed that *Dendrobium* tissue cultures showed a better response in Vacin & Went and Knudson's C media when compared with other media.

Street (1973) mentioned that White & Kordan were able to culture callus of lemon fruit and isolated vascular cambia in the presence of a sugar and mineral salts alone, while Goh (1972/3) observed an enhancement in the rates of protocorm proliferation and subsequent differentiation with the use of growth regulators. Earlier workers have incorporated various growth regulating substances in the culture media. Scully (1976), Street (1973) and Singh (1976) used naphthalene-

* Irawati was a Colombo Plan student doing a diploma course at the School of Ornamental Horticulture, Botanic Gardens, Singapore.

acetic-acid, while Steward and Button (1975) used 2,4 dichlorophenoxy-acetic-acid and 6-benzyl aminopurine. Gamborg (1975) found that thiamine was essential for the plant tissue cultures and Arditti (1974) modified Knudson's C by adding microelements to obtain better results.

To increase the availability of iron in the cultures, Murashige and Skoog (1962) added Na₂EDTA to FeSO₄ and noted that the resultant FeEDTA was beneficial to the tobacco tissue cultures.

Oxidation of endogenous phenolic compounds often does limit the successful growth of cultures as observed by Morel (1974) while working with *Phalaenopsis* tissue cultures and Goh (1972/3) while working with *Aranda*. Lindemann et. al. (1970) suggest that the tissue should be cut under sterile distilled water to reduce the oxidation of the phenolic compounds. Reinert and Bajaj (1977) found that immersion of the tissue of coffee in an isotonic medium and antioxidant further prevented the oxidation of phenolic compounds. The antioxidant used was ascorbic acid, diethyldithiocarbamate, L cysteine HCl, dithiothreitol or Cleland's reagent and glutathione or mercaptoethanol. Meiklejohn (1953) mentioned that ascorbic acid acts as an antioxidant by providing a supply of readily available hydrogen.

MATERIAL AND METHODS

Three orchid hybrids growing at the Botanic Gardens were chosen for the experiment, they are:

- a. *Oncidium* Goldiana (*Onc. flexuosum* X *Onc. sphacelatum*).
- b. *Dendrobium* Alice Spalding (*Den. tokai* X *Den. undulatum*).
- c. *Aranthera* Beatrice Ng var. Conference Gold (*Renanthera storiei* X *Arachnis* Ishbel).

Young shoots of the *Oncidium* and *Dendrobium* hybrids about 10 cm long and 15 cm stems of the upper part of *Aranthera* hybrids were cut from their parent plants. The leaves and sheaths were removed carefully from the shoots/stems, to expose the axillary buds. The apical part of the shoot was removed as only the axillary buds were used in this experiment.

The stem portion with the axillary buds was then surface sterilized using a 10% solution of commercial chlorox, for a period of 15 minutes. Explants about 4 mm cubes of tissue which included the axillary bud, were aseptically cultured in the different media. The buds were cultured in 60 ml test tubes which contained 4 ml of culture media.

To prevent the oxidation of *Aranthera* tissues, the excision of explants was done with the stems submerged in sterile ascorbic acid solution having a concentration of 2.5%.

Liquid Vacin & Went medium and Knudson's C medium with or without sucrose were used. Vacin & Went medium was modified with 5 ml/l of a stock solution containing 5.57 g FeSO₄·7 H₂O and 7.45 g Na₂EDTA per liter H₂O; 1 mg/l NAA; 1 mg/l Thiamine; 0.2 mg/l 2,4-dichlorophenoxy acetic acid and 0.2 mg/l Benzyl adenine to encourage proliferation of tissues, while Knudson's C media had microelements incorporated as suggested by Arditti (1974). The experiment was arranged in randomized block design with 10 replicates for each treatment.

Growth measurements were made every 2 weeks to determine an increase in the volume of the tissues. Observations were also made on the colour of the tissues and a colour range of 0-4 was used (0 for brownish/dead; 1 - whitish/yellowish; 2 - light green; 3 - green; 4 - dark green).

Each tube containing an axillary bud was placed on an agitator and subjected to 40 agitations per minute for 16 hours per day. The cultures were exposed to continuous illumination of about 200 foot candles and a temperature of 27°C.

RESULT

After a period of 11 weeks some of the *Oncidium* and *Aranthera* Beatrice Ng cultures survived. The percentages of survival are given in tables I and II.

TABLE I.
ONCIDIUM GOLDIANA

	Modified Vacin & Went	Modified Knudson's C
+ sucrose	20	80
- sucrose	70	66.6

TABLE II.
ARANTHERA BEATRICE NG VAR. CONFERENCE GOLD

	Modified Vacin & Went	Modified Knudson's C
+ sucrose	50	100
- sucrose	60	88.9

The surviving *Oncidium* Goldiana cultures did not show a significant difference between the 4 kinds of media both in size or colour (F test significant for 5% level).

However in *Aranthera* Beatrice Ng cultures there was a significant difference in their size and colour as given in table III.

TABLE III.
THE MEAN SIZE (MM³) AND MEAN COLOUR SCORE OF
ARANTHERA BEATRICE NG CULTURES

Treatment	Mean size (mm ³) *)	Mean colour score *)
Vacin & Went + sucrose	20 a	18. a
Vacin & Went - sucrose	32 ab	1.17 a
Knudson's C + sucrose	45 b	3.88 bb
Knudson's C - sucrose	41 b	4 bb

*) the same letter means no significant difference.

It can be seen that in respect of mean size (mm^3) and colour there is no significant difference in both media by adding or deleting sucrose, but they showed a significant difference in size (at 5% level) between Knudson's C media (with or without sucrose) and Vacin & Went media with sucrose.

However the two media (with sucrose or without sucrose) showed a high significant difference in their colour (F test of significance at 1% level).

CONCLUSION

It was observed that there is no significant difference by including sucrose in the two kinds of media tried for the three orchid hybrids. It is perhaps that the sucrose content of the coconut water used in these media was sufficient for growth of the orchid culture at that stage.

The significant different was found in *Aranthera* Beatrice Ng between Knudson's C media (with or without sucrose) and Vacin & Went with sucrose. Colour showed a high significant difference between the two media (with or without sucrose).

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